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**Prevalence of antibodies to feline parvovirus, calicivirus, herpesvirus, coronavirus, and immunodeficiency virus and of feline leukemia virus antigen and the interrelationship of these viral infections in free-ranging lions in east Africa**

Hofmann-Lehmann, Regina ; Fehr, Daniela ; Grob, Markus ; Elgizoli, Muhamed ; Packer, Craig ;  
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**Abstract:** While viral infections and their impact are well studied in domestic cats, only limited information is available on their occurrence in free-ranging lions. The goals of the present study were (i) to investigate the prevalence of antibodies to feline calicivirus (FCV), herpesvirus (FHV), coronavirus (FCoV), parvovirus (FPV), and immunodeficiency virus (FIV) and of feline leukemia virus (FeLV) antigen in 311 serum samples collected between 1984 and 1991 from lions inhabiting Tanzania's national parks and (ii) to evaluate the possible biological importance and the interrelationship of these viral infections. Antibodies to FCV, never reported previously in free-ranging lions, were detected in 70% of the sera. In addition, a much higher prevalence of antibodies to FCoV (57%) was found than was previously reported in Etosha National Park and Kruger National Park. Titers ranged from 25 to 400. FeLV antigen was not detectable in any of the serum samples. FCoV, FCV, FHV, and FIV were endemic in the Serengeti, while a transient elevation of FPV titers pointed to an outbreak of FPV infection between 1985 and 1987. Antibody titers to FPV and FCV were highly prevalent in the Serengeti (FPV, 75%; FCV, 67%) but not in Ngorongoro Crater (FPV, 27%; FCV, 2%). These differences could be explained by the different habitats and biological histories of the two populations and by the well-documented absence of immigration of lions from the Serengeti plains into Ngorongoro Crater after 1965. These observations indicate that, although the pathological potential of these viral infections seemed not to be very high in free-ranging lions, relocation of seropositive animals by humans to seronegative lion populations must be considered very carefully.

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# Prevalence of Antibodies to Feline Parvovirus, Calicivirus, Herpesvirus, Coronavirus, and Immunodeficiency Virus and of Feline Leukemia Virus Antigen and the Interrelationship of These Viral Infections in Free-Ranging Lions in East Africa

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While viral infections and their impact are well studied in domestic cats, only limited information is available on their occurrence in free-ranging lions. The goals of the present study were (i) to investigate the prevalence of antibodies to feline calicivirus (FCV), herpesvirus (FHV), coronavirus (FCoV), parvovirus (FPV), and immunodeficiency virus (FIV) and of feline leukemia virus (FeLV) antigen in 311 serum samples collected between 1984 and 1991 from lions inhabiting Tanzania's national parks and (ii) to evaluate the possible biological importance and the interrelationship of these viral infections. Antibodies to FCV, never reported previously in free-ranging lions, were detected in 70% of the sera. In addition, a much higher prevalence of antibodies to FCoV (57%) was found than was previously reported in Etosha National Park and Kruger National Park. Titers ranged from 25 to 400. FeLV antigen was not detectable in any of the serum samples. FCoV, FCV, FHV, and FIV were endemic in the Serengeti, while a transient elevation of FPV titers pointed to an outbreak of FPV infection between 1985 and 1987. Antibody titers to FPV and FCV were highly prevalent in the Serengeti (FPV, 75%; FCV, 67%) but not in Ngorongoro Crater (FPV, 27%; FCV, 2%). These differences could be explained by the different habitats and biological histories of the two populations and by the well-documented absence of immigration of lions from the Serengeti plains into Ngorongoro Crater after 1965. These observations indicate that, although the pathological potential of these viral infections seemed not to be very high in free-ranging lions, relocation of seropositive animals by humans to seronegative lion populations must be considered very carefully.

The most important viruses of domestic cats are feline calicivirus (FCV), feline herpesvirus (FHV), feline parvovirus (FPV), feline coronavirus (FCoV), feline leukemia virus (FeLV), and feline immunodeficiency virus (FIV). While these viruses are well studied in domestic cats, much less is known about their prevalence and importance in free-ranging lions (43, 44).

As no information is available on the occurrence of FCV, FHV, FPV, FCoV, and FeLV in lions of East Africa, it was the aim of the present study to investigate the prevalence of these infections in some lion populations living in Tanzania's national parks. In order to elucidate the possible importance of these infections in lions, essential features of these viruses are addressed briefly below.

FCoV occurs worldwide in domestic cats. Some isolates of FCoV are known to induce feline infectious peritonitis, while others cause very mild to fatal enteritis. Feline infectious peritonitis has been recognized not only in domestic cats but also in captive cheetahs, jaguars, lions, mountain lions, and leopards (9, 10, 16, 38, 40, 50, 51, 53). Cheetahs were found to be

dramatically susceptible to FCoV-mediated pathology, perhaps a consequence of genetic homogenization of the immune response in that species (12, 31). FCoV have been isolated from cheetahs that died during an epizootic at Wildlife Safari in Winston, Oreg. (11, 12, 38). Smaller wild felids kept in captivity, such as lynx, caracal, sand cat, serval, and pallas cat, have also been infected with FCoV (37, 39, 47, 53). In the populations of free-ranging mountain lions in California, approximately one-third of the animals had antibodies to FCoV (36), while none of the lions in Kruger National Park and only 3% of the lions in Etosha National Park were FCoV positive (43, 44).

FHV and FCV are agents causing mainly upper respiratory tract diseases. FHV causes rhinotracheitis and is therefore also referred to as feline viral rhinotracheitis virus; FCV in most instances is responsible for stomatitis, gingivitis, and circumscript lesions of the tongue. Clinical signs of rhinotracheitis have been described in captive cheetahs, jaguars, leopards, and mountain lions (3, 47, 48). Infectious agents were isolated from these animals and from a captive lion and characterized as FHV on the basis of typical cytopathic effect in cell culture, results of a neutralization assay, and appearance under an electron microscope (3, 48, 49). Most free-ranging lions had antibodies to FHV (67% in Etosha National Park, 91% in Kruger National Park), but none of them had antibodies to FCV (43, 44), while approximately one-fifth of the sera from

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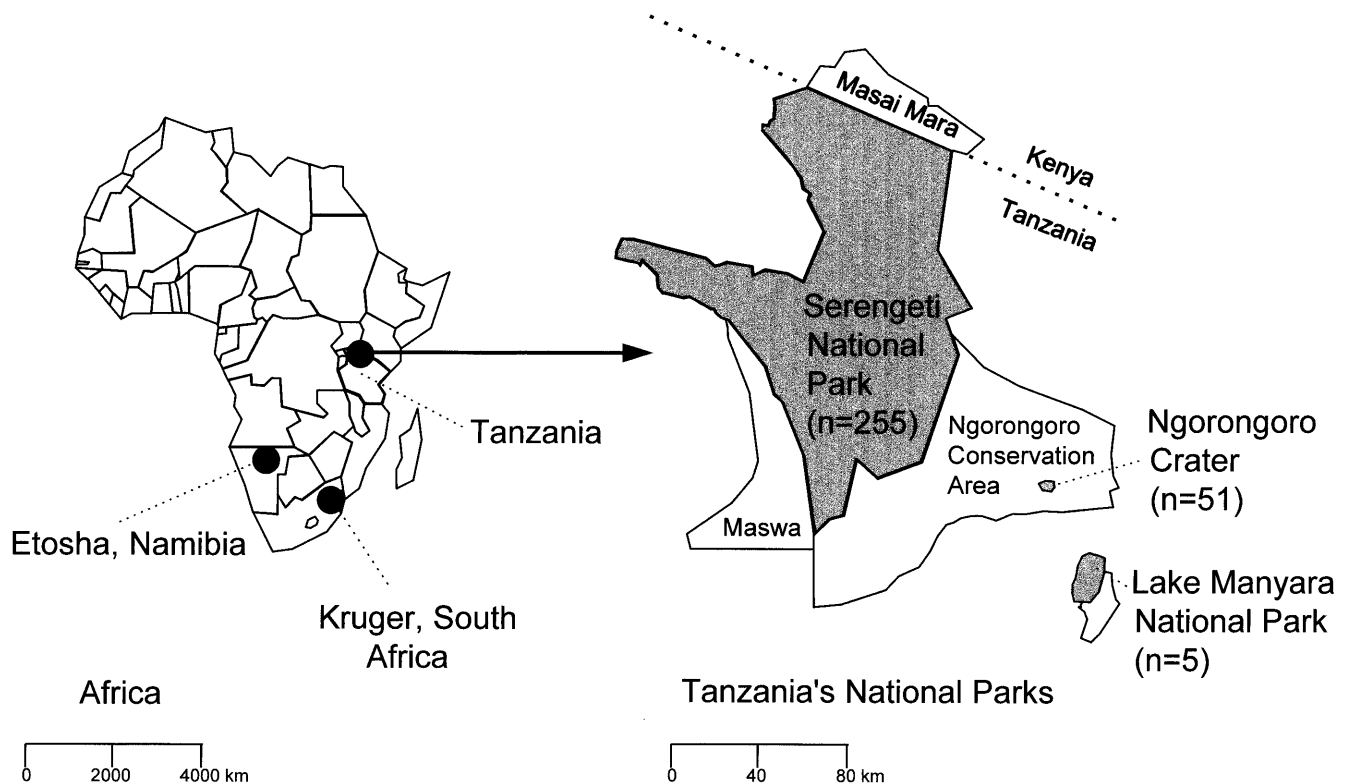


FIG. 1. Maps of Africa (left; scale approximately 1:105,000,000) and of Tanzania's National Parks (right; scale approximately 1:2,100,000). Serum samples were collected from lion populations inhabiting Serengeti National Park, Ngorongoro Crater, and Lake Manyara National Park. Numbers in parenthesis indicate the number of samples collected. In addition, Etosha National Park in Namibia and Kruger National Park in South Africa are indicated.

free-ranging mountain lions in California had antibodies to FHV and/or FCV (36).

FPV is highly contagious, inducing an acute disease characterized by leukopenia, fever, depression, dehydration, and diarrhea in domestic cats. In unvaccinated populations it has a high mortality rate. All members of the family *Felidae* are believed to be susceptible to FPV. In 1964, Johnson reported the isolation of feline panleukopenia virus from the spleen of a leopard cub born at Bristol Zoo (20). Subsequently, FPV was isolated from dead captive lions, panthers, snow leopards, and tigers (46, 47). The majority of the free-ranging mountain lions in California (93%) and the lions in Kruger National Park (84%) had antibodies to FPV (36, 43), while none of the free-ranging lions in Etosha National Park did (44). It was suspected that the lions in Kruger National Park had been infected by domestic cats, introduced some years earlier. However, the lions were not tested prior to this (43).

FIV infection is associated with immune suppression and opportunistic infections in domestic cats. FIV occurs worldwide in domestic cats. Antibodies to FIV have also been found in various zoo-kept wild felids (2, 5, 25). In addition, antibodies to FIV were detectable in cheetahs inhabiting East Africa, but not South or West Africa, in free-ranging pumas and bobcats inhabiting North America, and in leopards from Kruger National Park (2, 5, 33). FIV was found to be highly prevalent in free-ranging lions in East Africa (5, 33) and in Kruger National Park but not in India or Etosha National Park (5, 22, 25, 33). Lentiviruses, which are closely related to the immunodeficiency virus of domestic cats, were isolated from pumas and lions (6, 33). However, much less is known about the patho-

genicity and the biologic importance of the immunodeficiency virus in lions.

FeLV is known to cause neoplastic diseases and more frequently leads to an immunosuppression in domestic cats (15, 18). FeLV infection so far has never been described in free-ranging wild felids, with the exception of two confirmed reports: (i) two FeLV-positive European wild cats (*Felis silvestris*) trapped in northern Scotland that apparently caught the infection in the wild, however from an uncertain source (4), and (ii) a wild FeLV-positive cougar (*Felis concolor*), removed from a Californian college campus, which had supposedly preyed on domestic cats potentially infected with FeLV (19). Nevertheless, a serological survey in free-ranging mountain lions did not reveal any further evidence of exposure to FeLV (36).

It was the goal of the present study to determine prevalence and possible biological importance of these viral infections in free-ranging lions living on the Serengeti plains, near Lake Manyara, and in Ngorongoro Crater.

#### MATERIALS AND METHODS

Between 1984 and 1991, 311 serum samples were collected from free-ranging East African lions (*Panthera leo*) inhabiting Tanzania's Serengeti National Park ( $n = 255$ ), Ngorongoro Crater ( $n = 51$ ), and the Lake Manyara area ( $n = 5$ ) (Fig. 1). Twenty-four lions in Serengeti National Park and six animals from Ngorongoro Crater were sampled twice on different dates. The sera were stored at  $-20^{\circ}\text{C}$ . These serum samples had been collected by a team of researchers under the supervision of one of us (Craig Packer). Other results obtained from these sera were reported elsewhere (33). All sera were examined for the presence of antibodies to FCoV, FHV, FCV, FPV, and FIV and for the presence of FeLV antigen. Serological data were examined with respect to habitat, sex, and age of lions and year of blood collection.

**Detection of antibodies to FCoV.** Antibodies against FCoV were detected by

TABLE 1. Relationship between antibodies to FIV and antibodies to the other four feline viruses tested

FIV antibody status	No. of sera positive or negative for antibody to:							
	FCV		FPV		FHV		FCoV	
	Negative	Positive <sup>a</sup>	Negative	Positive <sup>b</sup>	Negative	Positive <sup>c</sup>	Negative	Positive <sup>d</sup>
Negative	13	5	10	8	0	18	15	4
Positive <sup>e</sup>	95	149	86	157	1	243	94	150
Yates $\chi^2$	6.35		2.13		0.149		10.26	
$P_{\text{chi}}$	0.0117		NS <sup>f</sup>		NS		0.0014	

<sup>a</sup> ELISA  $\delta\text{OD} > 0.100$ .<sup>b</sup> Hemagglutination titer  $> 10$ .<sup>c</sup> ELISA  $\delta\text{OD} > 0$ .<sup>d</sup> IFA titer  $\geq 25$ .<sup>e</sup> Western blot positive.<sup>f</sup> NS, not significant.

immunofluorescence assay (IFA), as described previously (24, 34), with transmissible gastroenteritis virus (Purdue strain). Serum samples were tested at dilutions of 1:25, 1:100, 1:400, and 1:1,600.

**Detection of antibodies to FCV.** Antibodies to FCV were detected by enzyme-linked immunosorbent assay (ELISA) and by IFA. CRFK cell monolayers were infected with FCV (strain 227/62) (7) and incubated until cytopathic effect reached about 60% of the cells. At the same time, mock-infected cells were grown and handled in an identical manner. Infected and mock-infected cells were scraped with a rubber policeman and lysed in Tris-sodium chloride consisting of 25 mM Tris-HCl, pH 8.2, and 150 mM sodium chloride, on ice, for 15 min. The lysates were treated with 0.1 M glycine-saline buffer (pH 9.0) at 10% lysate volume and incubated for 30 min at 37°C. Crude antigens were subjected to low-speed centrifugation at  $1,250 \times g$  at 4°C for 30 min. ELISA plates were coated with 100 ng of these antigens, diluted in coating buffer, per well (26). Serum samples were diluted 1:100 and tested by ELISA as described previously (26). As a conjugate, peroxidase-labelled rabbit anti-cat immunoglobulin G (Nordic Immunologic Laboratories, Tillburg, The Netherlands) diluted 1:2,000 was used. The results were given as the difference in optical density ( $\delta\text{OD}$ ) between FCV-infected cells and mock-infected cells. The same antigens were used for the IFA by the procedure described for FCoV with a modified serum dilution (1:10, 1:20, 1:40, 1:80, and 1:160).

**Detection of antibodies to FHV.** Antibodies to FHV were detected by ELISA and by IFA. CRFK cell monolayers were infected with FHV (CH strain) and incubated for 4 days. Mock-infected cells were grown at the same time and handled in the same manner. Cells were washed with phosphate-buffered saline (PBS) and placed on ice. Lysis buffer (50 mM Tris-HCl [pH 7.4], 100 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholic acid sodium salt, 0.1% sodium dodecyl sulfate) was added, and cells were scraped with a rubber policeman. A protease inhibitor cocktail containing 0.11 mM phenylmethylsulfonyl fluoride and 0.10 mM iodoacetamide was added for a further 15 min. When the suspensions were homogeneous, glycerol was added to a final concentration of 10% and the mixtures were allowed to stand overnight at 4°C. After centrifugation at  $1,750 \times g$  at 4°C for 20 min, supernatants were collected and dialyzed against 10 mM Tris-HCl (pH 7.4) containing 150 mM NaCl for 24 h at 4°C. The antigens were aliquoted and stored at  $-20^\circ\text{C}$ . The antigens were used for the ELISA (100 ng per well) and the IFA in the procedure described for FCV. In addition, 40 sera were tested by immunoblotting (Western blotting) with FHV-infected CRFK cells and mock-infected CRFK control cells according to procedures described previously for other antigens (23). The optimal amount of antigen per strip was determined previously by serial dilution of infected cell lysates.

**Detection of antibodies to FPV.** Antibodies to FPV were detected by a hemagglutination inhibition assay. Test and control sera were heat inactivated at 56°C for 30 min and treated with kaolin under conditions described previously (42) in order to eliminate nonspecific agglutinins. Samples were diluted in microtiter V-form wells in serial twofold dilution in 0.2 M glycine buffer (pH 9.0) starting at a dilution of 1:10. Serum dilutions (25  $\mu\text{l}$ ) were mixed with equal volumes of a FPV suspension containing four hemagglutination units. The virus was previously cultured on CRFK cells, frozen until use, and pretitrated the same day. Mixtures were incubated for 1 h at room temperature. Swine erythrocytes from sodium citrate-blood were washed with PBS three times. A suspension containing 1% erythrocytes was made in phosphate-borate buffer (pH 7.0) (15 mmol of NaCl, 11.1 mmol of  $\text{Na}_2\text{HPO}_4$ , 9 mmol of  $\text{KH}_2\text{PO}_4$  made up to 1 liter with  $\text{H}_2\text{O}$ , pH 6.7, mixed 1:1 with 150 mmol of NaCl, 50 mmol of  $\text{H}_3\text{BO}_3$  made up to 1 liter with  $\text{H}_2\text{O}$ , pH 9.4). Of this suspension, 50  $\mu\text{l}$  was added to each well, and plates were incubated for 90 min at room temperature. Antibody titers were read as the reciprocal of the endpoint dilution at which no erythrocyte agglutination was shown.

**Detection of antibodies to FIV.** Antibodies to FIV were detected by the Western blot technique as described previously (5).

**Detection of FeLV antigen.** FeLV p27 antigen in the serum was assayed by a double-antibody sandwich ELISA as described previously (27).

**Statistical methods.** Frequencies were compared by means of the two-tailed chi-square test with Yates' continuity correction, and multivariate analyses of seroprevalence were performed by logistic regression. Differences were considered significant at  $P < 0.05$ . Data on coinfection include only one sample from each individual.

## RESULTS

All results were analyzed with respect to habitat, sex, and age of lions and year of blood collection. No difference was found unless specifically stated.

**Prevalence of antibodies to FCoV.** All 311 serum samples were analyzed by the IFA to detect antibodies to FCoV. Forty-three percent of the lions had no detectable antibodies to FCoV, 50% of the animals had a titer of 25, 23 lions (7.4%) had a titer of 100, and the serum of one animal, a male lion originating from Serengeti National Park, yielded a titer of 400. Antibodies to FCoV were highly correlated with antibodies to FIV ( $P = 0.0014$  [Table 1]).

**Prevalence of antibodies to FCV.** The serum samples were analyzed by ELISA to detect antibodies to FCV ( $n = 310$ ). A significant difference was found to be related to the origin of the serum samples ( $P < 0.00001$ ): the majority of the lions in Ngorongoro Crater (82%) and all lions from the Lake Manara area showed no antibodies with reactivity to FCV ( $\delta\text{OD} = 0$ ). Only nine serum samples collected in Ngorongoro Crater yielded a  $\delta\text{OD} > 0$ . In contrast, 82% of the sera from Serengeti National Park produced antibodies to FCV (Fig. 2). The prevalence of antibodies to FCV characterized by a  $\delta\text{OD} > 0.100$  in the lions of Serengeti National Park increased with the age of the lions ( $P < 0.0001$  [Fig. 3]). Furthermore, the  $\delta\text{OD}$  increased with increasing age of the lions ( $P < 0.0001$ ; data not shown). In addition, 95 of the sera were analyzed by IFA. The results showed good agreement with the ELISA results (data not shown). Sera with a high  $\delta\text{OD}$  showed a specific fluorescence up to a dilution of 1:40. The presence of antibodies to FCV correlated with the presence of antibodies to FPV ( $P < 0.0001$ , even controlling for habitat and age of the lions [Table 2]). In addition, there was a strong relationship between antibodies to FCV and antibodies to FIV (Table 1). However, when the statistical analysis was corrected for age of the lions, this relationship disappeared: the relationship in animals younger than 3 years was not significant ( $P = 0.0903$ ).

**Prevalence of antibodies to FHV.** When tested by ELISA, antibodies to FHV were found in all samples except one ( $n = 310$ ). The frequency distribution of the antibodies from lions from Serengeti National Park resembled a normal distribution,

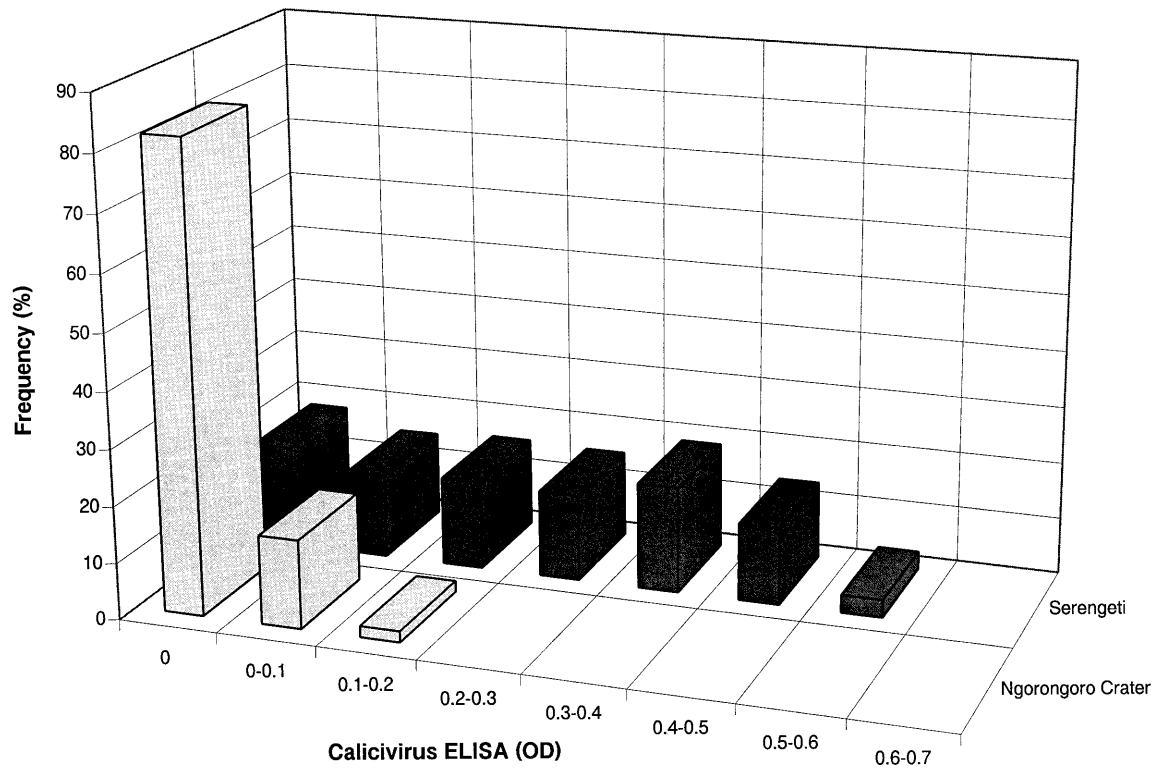


FIG. 2. Frequency distribution of antibodies to FCV detected by ELISA. The  $\delta$ OD indicates the difference in optical density yielded by antigen harvested from FCV- and mock-infected cell cultures. Lions are grouped by habitat. In Serengeti National Park, a significantly higher number of lions had antibodies to FCV than did lions in Ngorongoro Crater ( $P < 0.00001$ ).

with a maximum  $\delta$ OD between 0.4 and 0.5. In contrast, the sera from Ngorongoro Crater showed two maxima at a  $\delta$ OD of 0.3 to 0.4 and at a  $\delta$ OD of 0.7 to 0.8 (data not shown). The  $\delta$ OD increased with increasing age of the lions ( $P < 0.0001$ ;

data not shown). All sera tested by IFA ( $n = 28$ ) were positive at a serum dilution of 1:10. Some of the sera showed the specific fluorescence up to a dilution of 1:40. These sera were predominantly sera which had a high  $\delta$ OD in the ELISA. Two

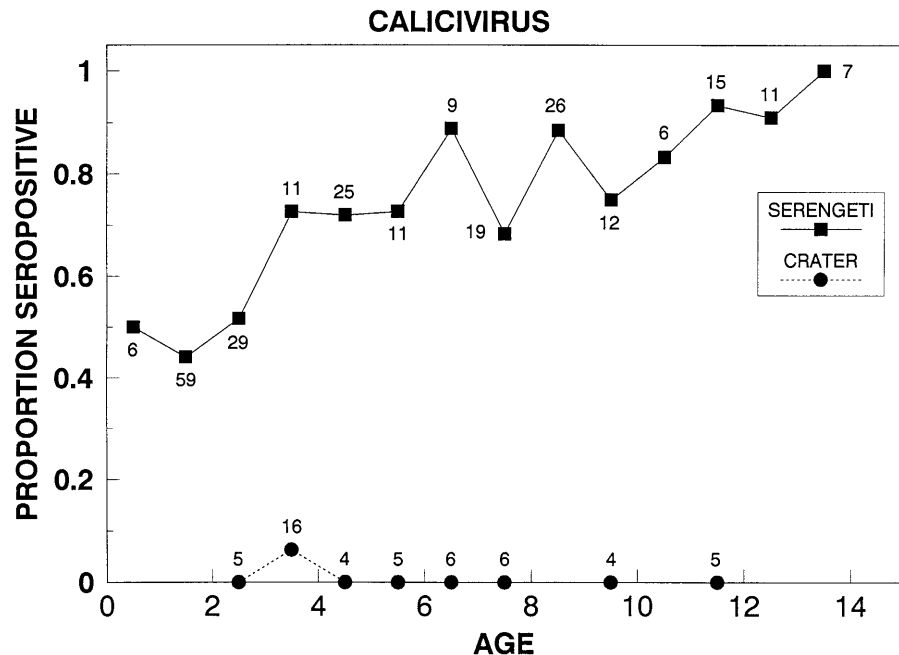


FIG. 3. Proportion of FCV-seropositive lions characterized as having an ELISA  $\delta$ OD of  $>0.1$ , grouped by age (in years). Numbers along the curve indicate the numbers of samples analyzed. Prevalence of FCV in lions of Serengeti National Park increased significantly with age ( $P < 0.0001$ ).

TABLE 2. Relationship between antibodies to FCV and antibodies to FPV

FPV antibody status	No. of sera positive or negative for FCV antibody	
	Negative	Positive <sup>a</sup>
Negative	80	34
Positive <sup>b</sup>	22	135
Yates $\chi^2$	6.35	
P <sub>chi</sub>	<0.00001	

<sup>a</sup> ELISA  $\delta$ OD >0.100.<sup>b</sup> Hemagglutination titer >10.

sera with a high  $\delta$ OD even tested positive when diluted 1:80. Western blotting resulted in the bands characteristic for FHV (13) when FHV-infected cells were incubated with ELISA-positive lion sera but not when mock-infected cells were incubated. These bands had molecular sizes of approximately 110, 75, 60, 50, 36, 33, 19, 16, and 14 kDa and corresponded well with the virus proteins vp5, vp7/8, vp9, vp12/13, vp18, vp19, vp21, vp22, and vp23 (13).

**Prevalence of antibodies to FPV.** Antibodies to FPV were detectable in 209 of 309 samples (68%) in the hemagglutination inhibition test. The titers ranged from 20 to >10,240. Low titers (<160) were found in 55% of all lions, while higher titers, which were found to correlate with protection in vaccinated domestic cats, were detected in 45% of the tested animals. A significant difference was related to the habitat of the lions ( $P < 0.00001$  [Fig. 4]): animals with high titers ( $\geq 160$ ) originated predominantly in Serengeti National Park (134 of 138 lions); only one lion from Ngorongoro Crater had an antibody

titer of 160, and all other samples yielded lower titers. A shift of titers was found when the data were analyzed according to the year of sample collection ( $P < 0.00001$  [Fig. 5]). In Ngorongoro Crater, no increase in the number of lions with high titers could be detected during the observation period. However, the one sample from a lion inhabiting Ngorongoro Crater which yielded a titer of 160 was collected in 1985. The five samples originating from lions in the Lake Manyara region had titers ranging from <10 to 5,120 and were all collected in 1987. A decline in titers was found when the data were analyzed according to the age of the lions ( $P < 0.0001$ ; data not shown). However, prevalence increased slightly with increasing age ( $P = 0.0248$ ; data not shown).

**Prevalence of antibodies to FIV.** Antibodies to FIV were found in 93% of the 44 sera tested from Ngorongoro Crater and in 91% of the 243 tested sera from lions inhabiting Serengeti National Park. No significant differences were related to sex or habitat of the animals. The virus was endemic. There was no measurable interaction between antibodies to FIV and the titer or the prevalence of antibodies to FPV and FHV (Table 1). However, antibodies to FIV were significantly more frequent in FCV-seropositive lions than in FCV-seronegative lions ( $P = 0.0177$ ) and were more frequent in FCoV-seropositive animals than in lions without antibodies to FCoV ( $P = 0.0014$ ; see above). The FIV status had no apparent effect on the lion's survival.

**Prevalence of FeLV antigen.** FeLV antigen was detected in none of the 311 serum samples tested.

## DISCUSSION

The aim of this study was to investigate the prevalence and importance of several feline viruses in free-ranging lions in

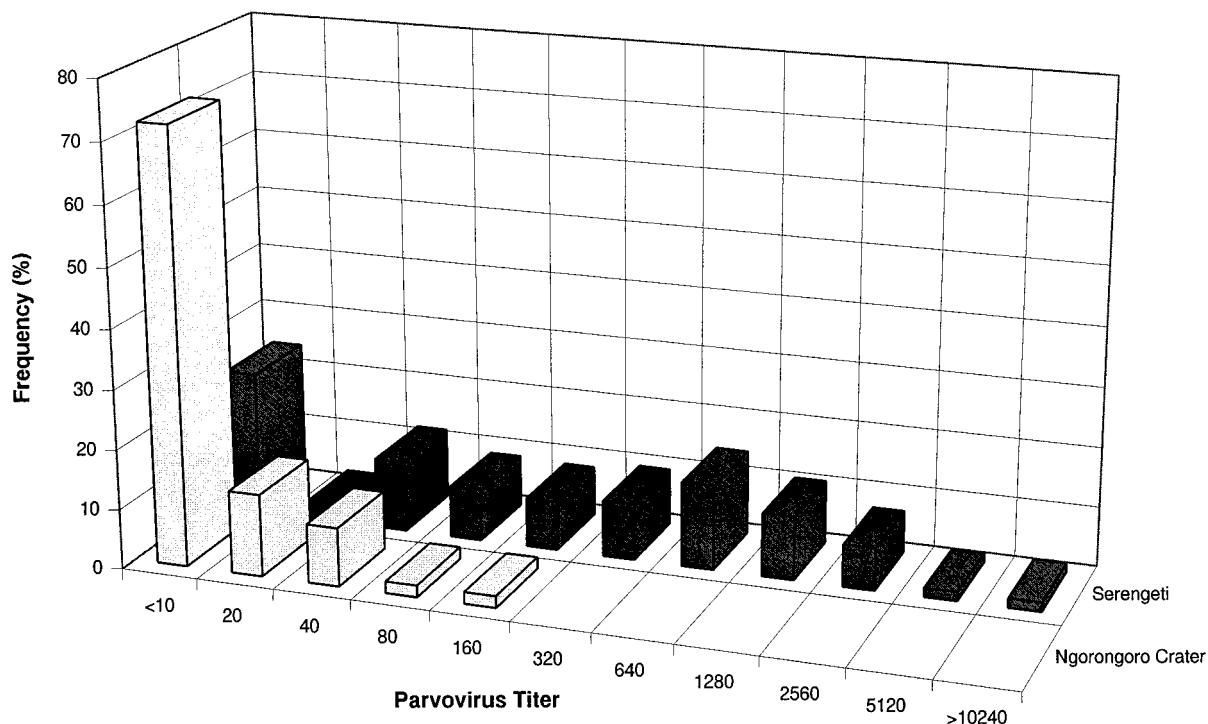


FIG. 4. Frequency distribution of antibody titers to FPV detected in a hemagglutination inhibition test. Lions were grouped by habitat. In Serengeti National Park, a significantly higher number of lions had high antibody titers to FPV ( $\geq 160$ ) than did lions in Ngorongoro Crater ( $P < 0.00001$ ). Lions from Lake Manyara National Park had titers up to 5,120 (data not shown).

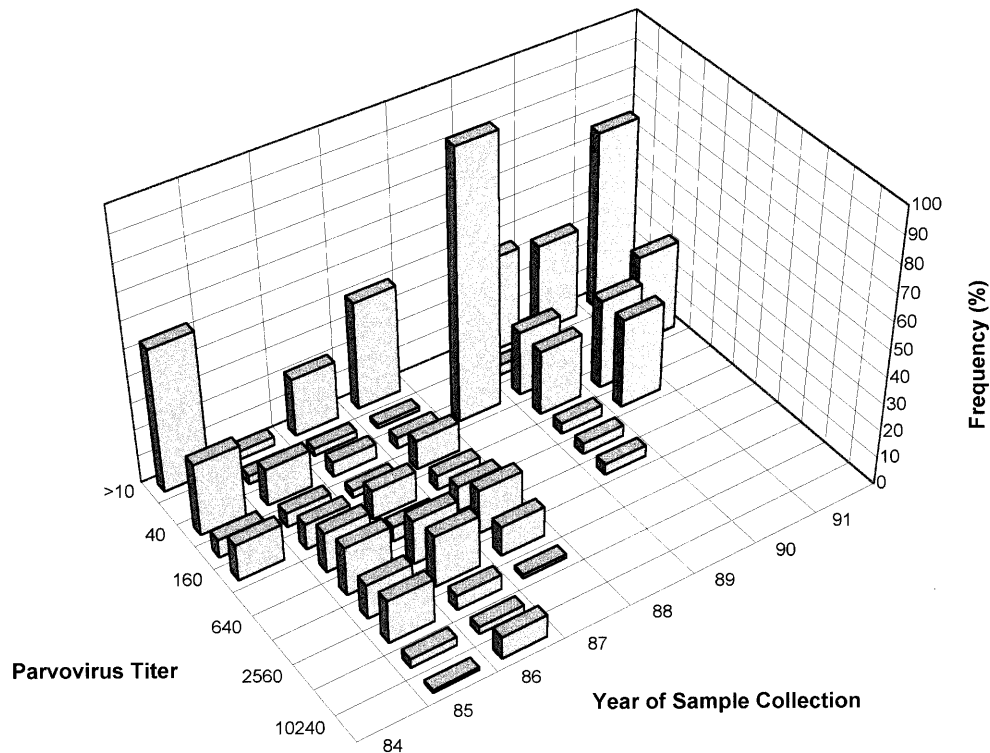


FIG. 5. Frequency distribution of antibody titers to FPV detected in a hemagglutination inhibition test with serum samples from lions inhabiting Serengeti National Park. Samples are grouped by year of collection. A shift of titers was found: increased titers in 1985 and 1986 and a decline thereafter.

habiting East Africa. Different serologic methods were used, such as ELISA, IFA, hemagglutination, and Western blot technique, in order to detect antibodies to FCV, FHV, FPV, FCoV, and FIV. FeLV antigen was measured by a sandwich ELISA. Serological data were examined with respect to habitat and sex of the lions and the year of blood collection.

**High prevalence and titers of antibodies to FCoV.** The IFA used in this study to detect FCoV allows specific fluorescence to be clearly distinguished from nonspecific signals as both infected and noninfected cells were present on the slide. The prevalence found in these lions (57%) is in accord with the

situation we see in domestic cats. No sex predisposition was seen. This result is also analogous to the situation found in domestic cats (1). The prevalence and the titers found were much higher than the previously reported prevalence and titers for free-ranging lions in other African national parks (43, 44) (Table 3, Fig. 1). In fact, the earlier studies reported antibodies to FCoV in none of the lions inhabiting Kruger National Park (43) and in only 3% of the lions in Etosha National Park (45). Whether these differences are due to assay conditions different from ours is unknown. Spencer and coworkers also used an IFA (43, 44). However, no information about the source and

TABLE 3. Comparison of the seroprevalence of FCV, FPV, FHV, FCoV, and FIV and of the prevalence of FeLV antigen in free-ranging lions from different parts of Africa

Area	No. of sera positive/no. tested for:					
	FCV	FPV	FHV	FCoV	FIV	FeLV antigen
East Africa						
Serengeti National Park	170/254 (67%) <sup>a</sup>	192/253 (75%) <sup>b</sup>	252/253 (99%) <sup>c</sup>	149/254 (58%) <sup>d</sup>	221/243 (91%) <sup>e</sup>	0/255 (0%) <sup>f</sup>
Ngorongoro Crater	1/51 (2%) <sup>a</sup>	14/51 (27%) <sup>b</sup>	51/51 (100%) <sup>c</sup>	28/51 (55%) <sup>d</sup>	41/44 (93%) <sup>e</sup>	0/51 (0%) <sup>f</sup>
Lake Manyara region	0/5 (0%) <sup>a</sup>	3/5 (60%) <sup>b</sup>	5/5 (100%) <sup>c</sup>	1/5 (20%) <sup>d</sup>	4/5 (80%) <sup>g</sup>	0/5 (0%) <sup>f</sup>
South Africa (Kruger National Park)	0/32 (0%) <sup>h</sup>	27/32 (84%) <sup>h</sup>	29/32 (91%) <sup>h</sup>	0/32 (0%) <sup>h</sup>	50/55 (91%) <sup>g</sup>	
West Africa (Etosha National Park)	0/66 (0%) <sup>i</sup>	0/66 (0%) <sup>i</sup>	44/66 (67%) <sup>i</sup>	2/66 (3%) <sup>i</sup>	0/66 (0%) <sup>j</sup>	

<sup>a</sup> ELISA  $\delta$ OD >0.100.

<sup>b</sup> Hemagglutination titer >10.

<sup>c</sup> ELISA  $\delta$ OD >0.

<sup>d</sup> IFA titer  $\geq$ 25.

<sup>e</sup> Western blot positive (more data will be presented separately [35a]).

<sup>f</sup> p27 antigen ELISA.

<sup>g</sup> Western blot positive (5).

<sup>h</sup> IFA titer = 10 (43).

<sup>i</sup> IFA titer = 10 (44).

<sup>j</sup> Recombinant p24 ELISA positive (45).

the properties of the virus was given. Assuming that the sensitivity and the specificity of the two assays are comparable, we conclude that the prevalence of FCoV antibodies in East Africa described here is indeed much higher than those in Kruger National Park and Etosha National Park. That we indeed did measure antibodies to FCoV is further supported by unpublished PCR (14, 17) results from one of our laboratories (D.F., R.H.-L., H.L., Clinical Laboratory, Department of Internal Veterinary Medicine, University of Zurich) in which we could demonstrate the presence of a coronavirus in some of the lion sera tested. Sequencing studies of this putative lion coronavirus are underway.

The possible disease spectrum associated with FCoV in lions is unknown. FCoV infection may not be of great importance to the clinical health of the free-ranging lions as there was no relationship between antibodies to FCoV and life expectancy.

**Correlation between antibodies to FCoV and antibodies to FIV.** There was a strong correlation between antibodies to FCoV and antibodies to FIV (Table 1). Such a correlation may be due to similar modes of transmission of the viruses, although this would be rather unexpected as in domestic cats FCoV infection is readily transmitted by mere close contact and does not require bites as in the case of FIV. Another possibility to explain this correlation would be cross-reacting antigens present in both virus preparations. Identical sequences of 5 to 10 amino acids have been found in infectious agents as unrelated as plasmodium, human immunodeficiency virus, and human influenza virus (28).

**Demonstration of antibodies to FCV in free-ranging lions.** Antibodies to FCV were detectable by ELISA in 70% of all lions. ELISA results were confirmed by IFA. Good accordance was found between ELISA and IFA. To our knowledge, this is the first evidence for the occurrence of antibodies specific for FCV in free-ranging lions. No antibodies to FCV were reported from lions in Kruger National Park or Etosha National Park (43, 44).

The prevalence of antibodies to FCV increased with increasing age of the lions (Fig. 3). In addition, the  $\delta$ OD measured by ELISA also increased with increasing age. The increased prevalence may be a sign of *de novo* infection of uninfected animals. The increase in  $\delta$ OD may reflect an increase in affinity of the FCV-specific antibodies in chronically infected or reinfected lions. ELISA procedures have been reported to detect high-affinity antibodies readily (8, 21). The same phenomenon of increased  $\delta$ OD with increasing age was also seen in the FHV data obtained by ELISA.

**Prevalence of antibodies to FCV in lions inhabiting different habitats.** Interestingly, high levels of antibody to FCV were found almost exclusively in lions originating from Serengeti National Park. Only very few animals from Ngorongoro Crater and none of the lions from the Lake Manyara region had high levels of antibody to FCV (Fig. 2, Table 3). Although these lion habitats are adjacent (Fig. 1), a spreading of contagious infections must not necessarily be expected between the Serengeti plains and Ngorongoro Crater. Ngorongoro Crater is a large volcanic caldera surrounded by a suboptimal habitat for lions. Therefore, only very limited emigration takes place, and no immigration of lions into the crater has been recorded since 1965 (35). Moreover, there is no direct connection between Lake Manyara National Park and Serengeti National Park. Therefore no exchange of animals is expected. Another explanation, but a rather unlikely one, is a decreased susceptibility to FCV of lions from Ngorongoro Crater. The lions of Serengeti National Park and Ngorongoro Crater reflect populations with quite different natural histories. Lions in Serengeti National Park represent a large outbred population and reflect a

high level of genetic diversity, while lions from Ngorongoro Crater have undergone a series of population bottlenecks (29, 30, 35, 52). It is not known whether this difference could explain a lower susceptibility to FCV in lions from Ngorongoro Crater, or on the contrary, whether it would lead to increased susceptibility, as has been reported in cheetahs for feline infectious peritonitis (32).

**Correlation between antibodies to FCV and antibodies to other viruses.** The occurrences of antibodies to FCV and antibodies to FPV were strongly correlated (Table 2). While a similar correlation between antibodies to FCV and antibodies to FIV (Table 1) could be explained by an increased prevalence of the two infections with increasing age, this was not the case with FCV and FPV. There are several other possible explanations for this correlation, such as (i) cross-reactivity of the two antigens, (ii) a similar mode of transmission of the two viruses, and (iii) higher risk of some lions for FCV and/or FPV infection due to certain risk factors, such as close contact with other species which could transmit the viruses to the lion populations. Of particular interest in this context could be close contact with humans and domestic cats. As cross-reactivity of antigens seems to be unlikely because different test principles were used, the correlation may rather be due to a similar mode of transmission or common risk factors.

**Antibodies to FHV.** Antibodies to FHV were found by ELISA in all of the samples except one. A similar situation was found in Etosha National Park and Kruger National Park, in which 67% and 91% of the lions were positive, respectively (43, 44) (Table 3). In order to confirm this very high prevalence of antibodies to FHV, 28 sera with low and high ELISA  $\delta$ ODs were also tested by IFA. All samples were IFA positive; titers varied from 10 to 80 and corresponded well with the results from ELISA. In addition, Western blot assays were established, revealing protein bands characteristic for FHV (data not shown). Different frequency distributions of the ELISA  $\delta$ OD from lions of the different habitats were demonstrated. The two peaks seen in Ngorongoro lions could be explained by the fact that, in four prides, relatively high antibody levels were present, while three other prides had low scores. It is striking that the interpride variation was not a function of the year sampled—samples were collected over several years—or of the animals' age. Therefore the high scores may not indicate epidemics at a specific time but could be due to different strains of FHV or to differences in the immune reaction of the lions involved.

**Prevalence of antibodies to FPV in different habitats.** Antibodies to FPV were detectable in the hemagglutination inhibition test in 68% of the samples tested ( $n = 309$ ). In domestic cats postvaccination titers  $>160$  were found to correlate with protection. In the lion population, 55% of the animals tested had a titer of 160 or higher. Whether this antibody titer induced by natural infection corresponds with protection is not known. Interestingly, the prevalence of high titers to FPV was much higher in lions from Serengeti National Park than in lions from Ngorongoro Crater (Fig. 4), suggesting an influence of the habitat. As in the case of FCV, the essential absence of FPV from lions of Ngorongoro Crater can be explained by the virtual absence of immigration of lions into the crater. Furthermore, the Serengeti plains are very rich in migratory prey animals. Therefore, lions sometimes move over large distances and thus encounter a greater number of other lions. This may favor spread of FCV and FPV in this location.

A decline in titers was observed with increasing age of the lions. In contrast to the ELISA system, as mentioned above for FCV, the hemagglutination system yields a higher titer for a serum with a low affinity but broader specificity than for a



serum with a high affinity and a very narrow specificity for only a few epitopes. Therefore, the decrease in titers with increasing age of the lions may reflect an increase in the affinity of the lion sera resulting from chronic infection or repeated reinfection.

**Increased titers of antibodies to FPV in 1985 and 1986.** It became clear that during the observation period the frequencies of titers to FPV fluctuated in the sera collected in Serengeti National Park (Fig. 5). On the basis of the observation that the titers were increased in 1985 and 1986 and declined thereafter, we speculated that a parvovirus outbreak must have occurred, which seemed transient and self-limiting. All samples from the Lake Manyara region were collected in 1987. Therefore, no follow-up over the years was possible in that area. No such fluctuation was found in the samples from Ngorongoro Crater, although interpretation was somewhat limited by the small number of samples collected in 1985 and 1987. However, these data support the observation that although the Serengeti plains and Ngorongoro Crater are adjacent, there was hardly any exchange of animals between these habitats. Natural barriers found around Ngorongoro Crater are able to protect animals from even highly contagious infections such as feline panleukopenia.

**Pathogenic potential of FIV.** The high prevalence of antibodies to FIV (91 to 93%) in lion populations inhabiting Tanzania's national parks came not as a surprise and confirms earlier findings (6). The present study adds new information in that the relationship between FIV and other infections can be studied. This point has been discussed above. It also allows speculation on the pathogenic potential of FIV in lions. If FIV infection had a significant pathogenic potential, it might be expected that coinfection with other viruses would lead to a more rapid clinical progression and therefore to a lower mean age of coinfecting animals. As there was no difference in the mean age of lions coinfecting with FIV and FCV and with FIV and FCoV (data not shown), it may be speculated that FIV in lions has a low pathogenic potential (unpublished data) (41).

In addition, there was no apparent relationship between the prevalence of antibodies to the five viruses and the survival of the free-ranging lions. More information on the effect of FIV infection on the health status and survival time will be published elsewhere (35a). It could be speculated that the impact of FCV, FPV, FHV, and FCoV infections on individual lions is not very big and that these infections do not endanger the lion populations, at least as long as there are no other aggravating cofactors.

**FeLV infection.** So far, there are only rarely reports of FeLV infection in wild felids, and most of them relate to captive animals. Only two of them are in association with free-ranging animals (4, 19). To our knowledge, this is the first systematic investigation of the occurrence of FeLV antigen in serum of free-ranging lions. From the fact that all of the samples were FeLV negative, we conclude that FeLV infection does not play a major role in free-ranging lions.

In conclusion, FCoV, FCV, FHV, FPV, and FIV, but not FeLV, are endemic in free-ranging lions inhabiting Serengeti National Park (Table 3). In addition, there is evidence for an outbreak of FPV infection between 1985 and 1987. However, the clinical relevance of this outbreak was probably minor, in that no significant changes in the mortality of cubs was registered between 1985 and 1987. Lion populations of different habitats in East Africa (Serengeti plains, Ngorongoro Crater, the Lake Manyara region) host different viruses (Table 3, Fig. 1). Natural barriers that prevent the exchange of lions also prevent the spread of even highly contagious infections such as FPV infection. In addition, different prevalences of antiviral antibodies were found in East African lions compared with

those of lions inhabiting Etosha National Park and Kruger National Park (Table 3, Fig. 1). Therefore, dislocation of lions by humans without consideration of the serological status might be very dangerous and must be considered very carefully.

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